Pinning Down Pheromones

Challenge

Many bacteria release chemicals called pheromones to monitor their population density. The bacteria use this information to regulate the transcription of certain genes — such as those that cause luminescence or biofilms. Scientists hope that by pinning down precisely how pheromones control gene behavior, they can learn how to manipulate them (with pharmaceuticals, for example) to prevent diseases in humans, such as cystic fibrosis, as well as diseases that affect our agricultural resources.

Argonne's Response

A team of Argonne bioscientists has been the first to observe the three-dimensional structure of a pheromone captured in the act of binding to a gene's regulatory element. The pheromone-DNA-transcription-factor complex they analyzed is from *Agrobacterium tumefaciens*, a bacterium that causes tumors in crops (Figure 1). Learning about the pheromone's structural convolutions and its binding sites helps scientists deduce how the pheremone functions on the molecular level. They can then hypothesize how similar systems may work in other pathogenic bacteria.

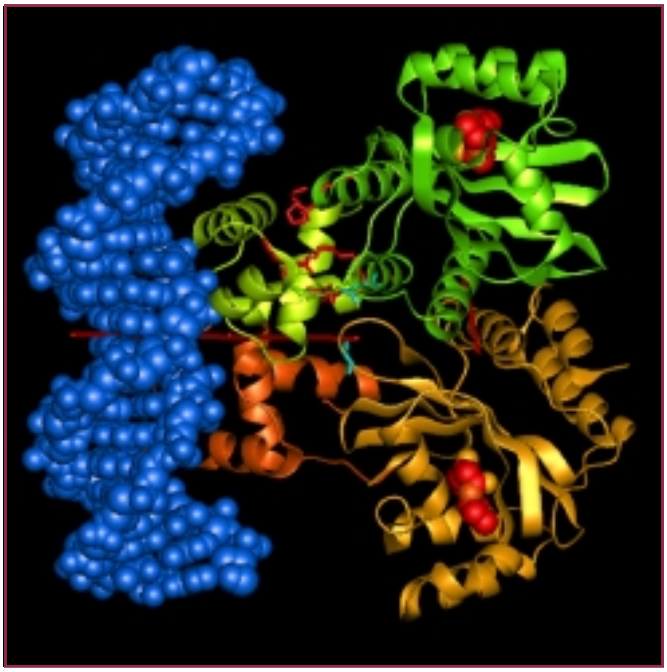

Figure 1. Three-dimensional visualization of the structure of a pheromone-DNA-transcription factor, as delineated by Argonne bioscientists using the Structural Biology Center at the Advanced Photon Source. Blue = DNA, red = pheromone, shades of red and blue = subunit-interaction sites and RNA polymerase activation sites on transcription factor protein.

Approach

The Argonne-solved structure is the first transcriptionally active complex combining pheromone with transcription-factor protein and DNA to be captured in three dimensions. Researchers used the world's most powerful x-rays — the Structural Biology Center beams at Argonne's Advanced Photon Source. The x-rays diffract from (and scatter off) the crystallized molecule, and the intensities of the diffracted beams are read by a computer that feeds these data into advanced structure determination programs (Figure 2). The structure was determined at a resolution of 1.66 angstroms — allowing scientists to detect the details of the structure. (To give some perspective: 10 million angstroms make up 1 millimeter.)

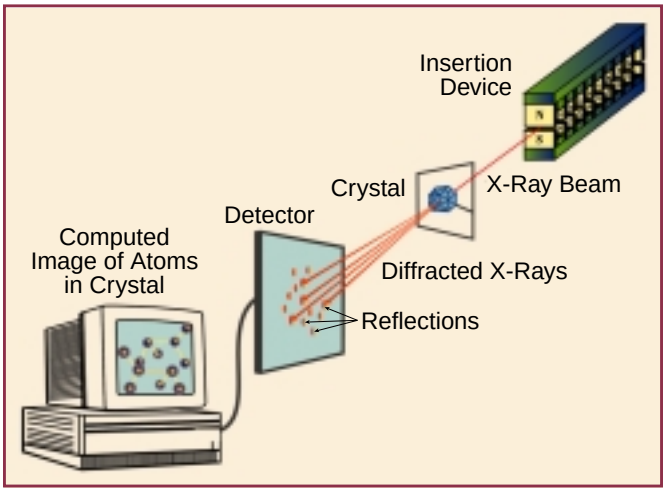

Figure 2. Diagram of x-ray-scattering analysis at Argonne's Advanced Photon Source, where scientists use the world's brightest x-ray beams to study nanoscale structures.

Results

By discovering the pheromone-binding site, Argonne scientists have determined that the *A. tumefaciens* pheromone works indirectly, by making the transcription-factor protein more stable and forming molecule pairs that are asymmetrical and activate gene transcription (Figure 3). They have also confirmed that the pheromone provides a necessary "scaffold" for formation of the molecule pairs, and that the asymmetry of the molecule pairs is likely to be significant in activating gene transcription. These structure-function relationships can be considered prototypes for similar pheromones.

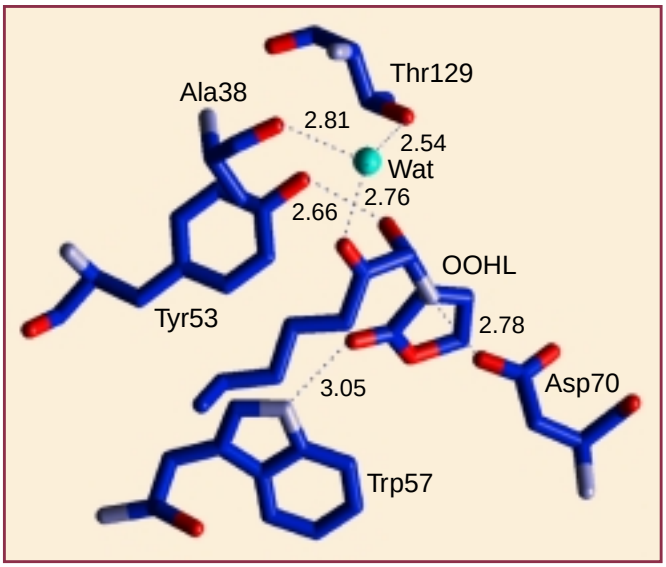

Figure 3. Argonne bioscientists determined the details of pheromone binding, at 1.66-angstrom resolution, of a TraR transcription-factor protein from A. tumefaciens *bacteria. The protein interacts with pheromone and a water molecule (light blue).*

Future Research

Work is under way to expand this research by:

- Looking at the binding sites of pheromone analogs,
- Creating structural mutations in the *A. tumefaciens* transcription factor to study their effects on DNA binding and on activation of gene transcription, and
- Determining the three-dimensional structure of the molecule pair complexed with RNA polymerase — catching it in the act of gene activation.

Collaborators

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